# Designing and implementing a rodent trapping study in Eastern Sierra Leone

## Preface

Unbiased estimates of small-mammal species presence and absence are required to model the association of species occupancy and land use type (Peterson et al. 2011; Stolar and Nielsen 2015). Previously available rodent trapping data from the Eastern Province of Sierra Leone was not amenable to these analyses as rodent sampling had generally been conducted as opportunistic surveillance to measure LASV prevalence or for cataloguing rodent species as part of development impact assessments (Fraser et al. 1974; Monath et al. 1974; Keenlyside et al. 1983; Barnett et al. 2000; Bonner et al. 2007; Decher, Norris, and Fahr 2010). Therefore, I designed and conducted a systematic, three-year rodent trapping study to investigate the research questions described in the introductory chapter to this thesis (@ref(introduction)) and reported in Chapters @ref(chapter3) and @ref(chapter4).

This methodology chapter describes current best-practice in small-mammal sampling in West Africa and novel methodological development. This chapter specifically, provides technical information on the data collection, sample processing and data cleaning steps. It is envisioned that documenting and providing evidence for decisions taken in designing this study will aid in the harmonisation and reproducibility of rodent trapping studies in the region.

## Trap selection

The use of commercial rodent traps was selected over locally produced traps. An important consideration for the choice of commercially produced rodent traps was the ethical capture of rodents. Typically, locally produced traps would take the form of “snap-traps”, these aim to incapacitate a trapped rodent with a spring powered mechanism that would break the neck of the rodent triggering the trap. However, these traps can injure the individual rather than killing them which could result in prolonged deaths and suffering for the trapped rodent (Hice and Velazco 2013). Further, setting the sensitivity of these traps is challenging and there would be differences in the sensitivity of each trap acquired resulting in different closure rates following triggering of the trap (Nicolas and Colyn 2006). Commercially produced traps have standardised sensitivity of closure mechanisms, the sensitivity of these mechanisms leading to a trap closure can be modified in the field if it is noted that the traps are not functioning as required. The standardisation of the closure method allows less biased comparison of detection rates between different individual traps and improves the reproducibility of the study design (Anthony et al. 2005). Finally, commercial scientific rodent traps do not aim to kill the trapped rodent which allowed us to adopt humane methods of rodent killing (i.e., cervical dislocation after anaesthesia), although arguments have been made that confining individuals within a trap only to euthanise them at a later point is less ethical than a quick death through a well functioning snap-trap (Nattrass, Stephens, and Loubser 2019).

The choice of commercial rodent trap would impact the detection of species. For example, the size of the trap may prevent larger rodents such as squirrels entering the trap and being caught (Harkins, Keinath, and Ben-David 2019). We therefore, selected the size of trap appropriate for our target population (7.62cm x 8.89cm x 22.86cm). Commercial traps are constructed as single capture or multiple capture devices. As we were interested in assaying caught rodents for acute infection it was important that we did not allow for individuals to come into contact under conditions in which viral transmission could occur and so we selected single capture traps. Several commercially available traps met these requirements, I selected the Sherman traps after reviewing the studies identified in Chapter @ref(chapter2) where these were the most commonly used commercial traps in West Africa. Local expertise in using Sherman traps already existed, which eased the implementation of the study protocol. A local stockpile of these traps and replacement parts was also available. An advantage of the Sherman traps was the ease at which they could be decommissioned during the days and activated at night to prevent unintended captures.

## Bait selection

The composition of bait used within traps will impact the species of small-mammals trapped. No studies in food preference have been conducted in the rodents and shrews of Sierra Leone. However, elsewhere in sub-Saharan Africa studies on diet composition of *M. natalensis*, *R. rattus* and *M. minutoides* have been conducted, in both wild and captive populations which observed that these species consume raw crops, insects and processed foods, particularly when detected within human homes (Iwuala, Braide, and Maduka 1980; Mbise, Kilonzo, and Kinabo 1995; Odhiambo et al. 2008; Mulungu et al. 2014; Mlyashimbi et al. 2018).

Studies conducted in the region did not generally describe the composition of bait that is placed in traps. Bait that has been previously shown to be effective at attracting individuals of species of interest included peanut paste, palm oil and dried fish (Fichet-Calvet et al. 2005; Mariën et al. 2017; Bonwitt et al. 2017). No studies have been conducted in the region comparing the success of different bait compositions and so during the piloting the study I compared the trap success using a bait comprised of peanut paste, palm oil and dried fish with one of peanut paste, palm oil and goat meat. No difference in trap success was observed between these two baits and so dried fish was used throughout the remainder of the study. Bait ingredients were purchased locally at each study site and combined following a standard recipe (10 parts peanut paste, 1 part palm oil, 1 part dried fish). Traps were re-baited each day to ensure the amount of bait within the trap was equivalent between trap nights.

The bait also attracted insects and off-target animals (i.e., frogs) to the traps. Off-target species were released at the site of the trap during the re-baiting process. Insects attracted to the traps potentially had an impact on the detection of insectivorous shrew species within our study region (i.e., *Crocidura sp.*). Shrew species were not considered off-target small mammals as the spatial interaction between these species and rodents within our study setting was something we wanted to observe. Previous studies have shown that shrews are potentially infected with LASV although their ability to maintain transmission is not known (Fichet-Calvet et al. 2007; Kerneis et al. 2009; Kenmoe et al. 2020). Therefore, sampling shrew populations in conjunction with our rodent primary targets would improve understanding of the small-mammal community potentially involved in LASV transmission.

A further consideration in the selection of bait is the availability of alternative food sources within the environment of the trap. For example, in areas of human habitation or agricultural settings the high availability of alternate sources of food may lead to a reduced need for rodents to enter traps, overcoming their fear of novel environments (i.e., neophobia) (Stryjek, Kalinowski, and Parsons 2019). We did not measure the availability of alternate food sources and so were unable to disentangle the potential impact of this on the probability of detection of a rodent within a trap.

## Village study site selection

Lassa fever is endemic in the region surrounding Kenema in Eastern Province, Sierra Leone. To identify village study sites where human infections with Lassa fever had previously been identified from I met with the provincial Paramount Chief and regional elders. The aim of this meeting was to identify potential village study sites that had previously reported Lassa fever outbreaks and locations where no outbreaks had been reported. I aimed to select villages representative of those with known and no prior outbreaks to ensure that we were not conditioning our sampling based on expected Lassa fever outbreaks. The inclusion of two villages where Lassa fever had not previously been reported (Seilama and Baiama) and two villages where it had been reported (Lalehun, Lambayama) would help to ensure that any differences between rodent communities within these settings would be due to land use type rather than LASV presence among rodents.

It was also important to ensure that village communities would support our longitudinal study. The rodent sampling was designed to be a multi-year study and so we needed to select village study sites that would allow repeated visits for activities that could be associated with stigma among the local community (i.e., sampling wildlife for viral pathogens). To optimise the support we received from local communities the study and rodent sampling protocol were described in detail to the local chieftain and the village committee. All concerns about the risk to the local community from our activities and the impact on their activities were addressed and we committed to employing community members during our sampling activities. An exit interview was agreed where we would share our findings with the villages and this was conducted during February 2023. At these interviews we explored the impact of our sampling activities, all villages reported no adverse effects of our trapping activity and were keen to continue in the study were additional funding acquired.

## Trap site selection

To estimate the occurrence of rodent species across a land use gradient in our study region we aimed to conduct rodent trapping representative to the land use type in Eastern Sierra Leone. I produced land use type rasters of the region from remote sensing data which were ground-truthed to observations made in the field (Jung et al. 2020). Remote sensing data has several limitations in this region of West Africa which complicated this approach. The transient nature of agriculture practices in the region (i.e., slash-and-burn) makes it challenging to train classification models to identify land used for agriculture, when compared to classification outside of West Africa (Thenkabail 1999; Kusimi 2008). Secondly, the mixed use of land within the region, for example crops interspersed within plantations or plantations in forest settings can lead to the misclassification of land use based solely on remote sensing (Alabi et al. 2022). Using the combination of remote sensing and ground-truthed observations I selected trap sites representative of village and agricultural land use along with less disturbed forest settings. To approximate representativeness of trapping effort weighted to the area of land within a radius of 20km2 of the village study sites we implemented one site in forest, four in agriculture (two in proximal and two in more distal settings) and two within villages (one in outdoor and one in indoor settings).

The exact selection of trapping grid locations within the village study sites was guided by several factors. First, it was important that the trapping grids could be accessed in all weather conditions. Sierra Leone has a dry and rainy season with substantial amounts of precipitation in the rainy season degrading roads and tracks making access to remote sites challenging. Second, trapping grids needed to be accessible during all trapping sessions. Areas of forest around villages may be locations of special community importance, for example, grave-sites or locations of ceremonies for secret societies (Lebbie and Guries 1995; Martin et al. 2011; Ménard 2017). We ensured that we selected sites that would be accessible throughout our study period through close collaboration with our village communities. Finally, we wanted to ensure that study sites would not be converted to different land use types during our study (i.e., conversion of forest to agriculture or agriculture left to fallow), we therefore discussed planned sites with the community to ensure our trapping grids were not planned for significant conversion.

## Trap location

Once trap sites had been selected we identified the structure of the trapping grids that would be placed within these sites. It was important for the interpretation of the association of rodent occupancy with land use type that the local habitats of the traps did not substantially vary across our trap sites. In addition as we aimed to infer contacts between individually trapped rodents based on the proximity of the locations at which they were detected it was vital that trapping effort was evenly distributed across the entire area of the study site. We therefore, decided on a grid structure of traps over alternate approaches including trap lines and trap webs.

Each trapping grid was comprised of 49 traps, the number of traps placed within a grid was directed by practical considerations, including the number of available traps and the time requirement to set-up, record and bait the traps. We placed individual traps within a grid structure with 7m between each trap to sample an area of 2,401m2 within each grid. The exact habitat type and geographic location of each trap was recorded at each study session, photographs were used to document the habitat type where it did not meet the pre-specified options in the data collection tool. Traps that successfully captured a rodent during a study night were removed from the grid - after marking the location with a flag - for processing, before being returned to the same location during re-baiting for subsequent trapping nights.

## Repeated trapping

The rodent trapping study was designed to repeat sampling of the same locations at multiple timepoints. Sampling occurred four times annually, the selection of this was guided by several factors. As this study was designed in the context of investigating LASV transmission, it was important to ensure that we would be able to detect local transmission were it to occur in our study locations. The lifespan of rodents in these settings is expected to be less than one year, therefore longer time periods between visits may have resulted in a different population of rodents being sampled than those alive at the time during pathogen transmission (Safronetz et al. 2022; Leirs, Verhagen, and Verheyen 1993). In addition, evidence from Guinea and Nigeria suggests a seasonal component to LASV incidence among rodents and humans (Fichet-Calvet et al. 2007; Olayemi et al. 2016). To increase the probability of detecting pathogen transmission were it occurring high frequency trapping would be preferred, however, as we were using removal trapping we did not want to adversely affect the population structure of our small-mammal populations. Three-monthly sampling was used to balance these conflicting demands, based on a study investigating the impact of removal trapping on rodent abundance in Guinea (Mari Saez et al. 2018).

To allow comparison between sampling at grid sites during repeated visits we recorded the GPS coordinates of the outer boundaries of the trapping grid. It was not feasible to ensure that individual traps were placed at the exact locations of traps at previous visits and so the exact locations of traps varied across individual visits. To support subsequent spatial analysis of the trapping data we aggregated individual traps within grid cells. We constructed a regular grid across the trapping grids with each cell measuring 49m2. Individual traps were allocated to a single cell with the centroid of these cells providing a standardised location of traps placed within these cells. Where multiple traps were placed within a single cell the traps were aggregated (Appendix @ref(trap-harmonisation)).

The repeated measures of detection in the presence of imperfect detection were used to model occupancy of small-mammal species across a land use gradient, as described in Chapter @ref(chapter3). It was expected that the probability of detection of a species within a land use type would not be constant across repeated study visits (Springer et al. 2016). To account for this we collated data on factors that may be causing a difference in detection rate (i.e., light level, precipitation and trapping effort). For example, it has been suggested that rodents and shrews may have variable activity, and therefore detection probabilities, in different conditions of ambient light caused by local predation pressure (Paise and Vieira 2006; Williams et al. 2014). We were unable to accurately measure light levels at individual trap locations and therefore used a proxy measure of moon fraction (i.e., proportion of full moon) during the sampling visit to approximate ambient light levels. This approach may not have adequately measured light level at the exact time and locations of rodent activity. For example, within forest settings light levels may be lower than would be expected due to cover by foliage. Cloud cover may also have impacted ambient light, particularly in the rainy season where cloud cover may be greater than in the dry season where the same moon fraction may lead to lower ambient light at ground level.

## Morphometric measurements and identification

Data were obtained for all individuals on standard morphometric measurements including weight and length of body, head, hind foot etc. These measurements, alongside more general characteristics, including pelage colour supported classification using the produced taxonomic key (@ref(taxonomic-key)). Morphometric measurements and reproductive status were also used to age stratify individuals into juvenile or adult classes. Eye lenses were obtained from rodents with the aim to further classify by age using standardised eye-lens weight growth charts for species where this was available (Hardy, Quy, and Huson 1983; Fichet-Calvet et al. 2008).

Several rodent and all shrew species were not able to be differentiated based on the taxonomic key, for these species additional molecular identification were required. Sequencing of Cytochrome b has previously been used for rodent and shrew species in West Africa and has been shown to be able to discriminate between these cryptic species (Bradley and Baker 2001; Lecompte et al. 2002). While some rodent species could be unambiguously classified using the taxonomic key, molecular sequencing was performed on all individuals to improve confidence in field-based identification and reduce the impact of misclassification on subsequent analysis.

## Sample collection and processing

Samples were collected following established protocols for rodents and shrews (Mills et al. 1995; Fichet-Calvet 2014). Concerns around releasing potentially infectious rodents back into the village communities led us to euthanise all captured individuals. Blood sampling could thus be performed through cardiac puncture as captured individuals were euthanised. Cardiac puncture should lead to greater volumes of blood sampled compared to non-lethal techniques such as capillary eye blood and tail vein sampling (Mills et al. 1995). However, for very small individuals insufficient volumes of blood are obtained via cardiac puncture, for these we collected blood on filter paper and obtained heart tissue samples. Filter paper samples are expected to have lower sensitivity for antibody and viral detection which may be related to methods of elution from the filter paper (Amini et al. 2021; Soubrier et al. 2022).

Samples were stored in appropriate storage media (i.e., formalin or ethanol) and placed in cool boxes on ice packs to maintain a cold chain. Ideally samples would be stored in liquid nitrogen in the field, however, this was not feasible in this region of Sierra Leone where there was no availability of liquid nitrogen. To minimise the time samples were stored outside of an effective cold chain we partnered with Panguma District Hospital and Kenema General Hospital to store samples in -20°C at the end of each day of sampling. Long term storage of samples was at our local collaborators lab based at Mercy Hospital, Bo, here samples were stored in -20°C freezers. At the Mercy Hospital location back-up power banks were able to maintain a cold chain during the daily episodes of disrupted electricity supply. However, at several points during our study municipal power was not available for prolonged periods, it is likely that this led to samples temporarily increasing above -20°C, although it is not expected that this would have a significant impact on the degradation of antibodies prior to analysis (Amini et al. 2021).

All sample processing and analysis was conducted in-country in Sierra Leone. A single step of the analysis pipeline could not be conducted in Sierra Leone as there were no Sanger sequencing machines available to the research team, therefore PCR products were shipped to Germany for sequencing. Conducting all laboratory analysis in Sierra Leone was a deliberate choice to improve local ownership of the research project and to consolidate the knowledge and skills of the local workforce in the processing of biological samples. Additionally where possible students from local higher education institutes were included in sample analysis for training purposes. All samples remain in Sierra Leone for future use by the local research community and international collaborators.

## Data collection and processing

Data collected in the field were recorded in real-time. Initially paper data entry forms were designed to capture information on trap locations and captured rodents, these forms were trialled during the initial sampling visits and improved following feedback from the field team. These forms required digitisation which could lead to data entry errors and delays in reviewing for accuracy and completeness. To counter this and allow for remote support in data entry a digital data capture tool was subsequently adopted. The OpenDataKit (ODK) is an open-source software that has been constructed to support offline data entry through powerful forms which allow multi-media data entry (Open Data Kit 2023). The ability to store data locally when offline for it to be subsequently uploaded to dedicated servers when a network connection is found is particularly valuable when conducting research in remote regions.

Digital data entry forms were produced for setting up of traps, checking traps and recording captured small-mammals. The trap setup form allowed photographs of trap locations to be collected alongside description of trap habitats and the coordinates of the placed traps. The trap-check form allowed rapid entry of the status of each trap the following morning, recording whether traps were missing bait (i.e., sensitivity of trap closure may have been too low), closed but empty (i.e., sensitivity of trap closure may have been too high) or contained a small-mammal or other animal. Finally, the small-mammal capture form included information on the trap in which the individual was captured, photographs of the individual, identification based on the taxonomic key, morphometric measurements and sample acquisition. Together these forms allowed traceability of samples from individual rodents to be traced back to the location in which they were trapped through an auditable records trail.

The data entry forms could be completed on any smart device, initially we provided the field team with tablets for data entry but they preferred to use their own phones for data entry as they were more portable and faster to use. The forms were accessed through a mobile ODK application (ODK Collect) with users given access to the required forms (ODK Collect 2023). Data were encrypted locally after form completion and sent securely to an ODK Central server hosted by the London School of Hygiene and Tropical Medicine. Data were then downloaded from this server using the ruODK R client for the ODK Central API (Mayer 2023). The instant availability of data allowed real-time support to the field team to be provided.

Substantial data processing and cleaning were required to correct inaccuracies in data entry. To facilitate this, scripts to perform data cleaning were written in R which allowed the original data to remain unmodified while also producing a reproducible and automated pipeline for data processing. The majority of data inaccuracies were associated with entry of GPS coordinates which could be remedied by asking the field team to take photographs of the GPS recorder, other common errors included misallocating study grid numbers or visit numbers which could be corrected using other sources of information (i.e., coordinates of traps or date of form entry). All code to perform data processing and cleaning are available in a GitHub repository that reproduces the data used for analysis from the raw data uploaded to ODK Central (Simons 2022). This improves the reproducibility of the thesis through a transparent data and analysis pipeline. The R code to clean the raw data is commented and fully documented within the GitHub repository which will support its re-use. Finally, the availability of this R code and data entry forms can be used by other researchers to aid data collection in small-mammal sampling studies.

## Conclusion

This chapter summarises the decisions taken to design the rodent trapping study that generates the data for the following chapters. I have described the steps in selecting the traps, bait and locations in which the study was conducted alongside challenges in making these decisions. I go on to describe approaches taken throughout to improve confidence in generated data and to produce a transparent pipeline from data acquisition through to data analysis which I hope will be of benefit for researchers within the field of disease ecology and beyond.

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